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(54) Title: IMPLANTABLE BIOCOMPATIBLE ANIMAL TISSUE

(57) Abstract: Undesired agents reducing biocompatibility are selectively and substantially completely removed from tissue of animal origin intended for implantation in medical devices. Ambient pressure and temperature of a supercritical fluid solvent comprising carbon dioxide are adjusted to selectively remove one or more undesired agents from animal tissue perfused by the solvent. Calcification tendency of implantable tissue of animal origin is estimated as directly proportional to amounts of calcific agents removable from the tissue. Tissue engineering matrices are prepared from tissues of animal origin by selective and substantially complete removal of undesired agents incorporated in the tissue.

Implantable Biocompatible Animal Tissue

Description

Background Art

This invention relates generally to devices intended for implantation into patients. More particularly, this invention concerns implantable medical devices comprising animal tissues treated to improve the biocompatibility of said tissues after implantation.

Implantable medical devices incorporating tissue of animal origin have become critical in the management of a variety of human diseases. Bioprosthetic heart valves, for example, may incorporate stabilized (i.e., crosslinked or otherwise fixed) animal tissue in the form of porcine heart valve leaflets or bovine pericardial tissue. Such valve leaflets are favored to replace damaged human heart valves in certain patients because they mimic the natural valve action of healthy human hearts. But stabilized porcine heart valves and bovine pericardium, as well as other bioprosthetic tissues of animal origin, have demonstrated certain biocompatibility problems after implantation. Post-operative calcification can limit the useful life of any such bioprosthesis by interfering with its normal function. Such valves may also be cytotoxic because of residues of the crosslinking agent, such as glutaraldehyde, remaining in the tissue. Serious and costly complications are possible, including the need to remove and/or replace the implanted device.

It is believed that cellular debris, residual tissue stabilization agents (e.g., crosslinking catalysts), tissue phospholipids and/or other relatively low-molecular-weight compounds naturally present in animal tissue contribute to reduced biocompatibility. One particular problem is post-implantation calcification. Consistent with this theory, many anticalcification tissue treatments have been reported. Organic solvents such as ethanol, dimethyl sulfoxide and glycerol are used to remove lipids from tissue, but the solvents themselves must then be removed prior to implantation. Following their recovery, such solvents must be completely decontaminated for reuse or eliminated as industrial waste.

The present invention includes methods for anticalcification treatment incorporating simplified solvent recovery and decontamination. Animal tissues treated by such methods and implantable bioprosthetic medical devices comprising tissues having improved biocompatibility are also included in the invention.

Disclosure of Invention

The present invention comprises methods and apparatus relating to improving the biocompatibility of tissues of animal origin incorporated in bioprosthetic medical devices for

implantation in humans. Such improved biocompatibility is achieved according to one embodiment of the present invention through selective and substantially complete removal from the animal tissue of each of a heterogeneous group of calcific agents that may be incorporated in the tissue. Such calcific agents include, for example, cellular debris, residual tissue stabilization agents (e.g., crosslinking catalysts and/or other materials used for tissue fixation), tissue phospholipids and/or other relatively low-molecular-weight compounds naturally present within and/or on animal tissue.

According to the present invention, selective removal of undesired materials such as calcific agents of the above heterogeneous group may be accomplished by perfusing the animal tissue with a supercritical fluid solvent comprising supercritical or near-supercritical carbon dioxide (SCO2) or other allogenic supercritical fluid. SCO2 is insoluble in water but can be a powerful solvent for lipids, oils and other small molecular weight organic compounds. By adjusting its solvating power through known methods such as changes in temperature and/or pressure (as disclosed in, e.g., U.S. Patent No. 5,533,538, incorporated herein by reference), a supercritical fluid solvent comprising SCO2 can be made to selectively remove calcific agents on or within the animal tissue. Iterative applications of such a supercritical fluid solvent, with appropriate adjustment of solvating power between applications, can be used to selectively and substantially completely remove a wide variety of undesirable agents from animal tissue. Where necessary, a supercritical fluid solvent of the present invention may additionally comprise one or more cosolvents and/or surfactants. Cosolvents generally aid removal of otherwise insoluble calcific agents from animal tissue, while surfactants generally aid penetration of animal tissue by the supercritical fluid solvent.

Supercritical carbon dioxide is commercially used to extract flavors and oils directly from seeds and other agricultural feed materials. Supercritical fluids (including fluids comprising carbon dioxide) have also been described as useful for treating bone tissue to remove fat and protein material, and for extracting lipids, proteins, nucleotides, saccharides and other components from animal tissues, cells and organs. See, e.g., U.S. Patent Nos. 5,725,579 and 4,749,522, both incorporated herein by reference. Applicants, however, are unaware of any prior disclosure of the use of adjustable solvating power in a supercritical fluid solvent to prepare animal tissues having improved biocompatibility for implantation in bioprostheses as described herein.

Another aspect of the present invention relates to recovery of selected calcific agents removed from animal tissue. The amounts of such agents recovered at any point in the process of making biocompatible animal tissue furnish a useful guide as to how much additional treatment is

required for the tissue in question. Knowing such amounts also allows estimation of the (directly proportional) calcification tendency of the corresponding animal tissue. This aspect of the present invention provides a means for assuring uniform quality in implantable bioprostheses, notwithstanding the naturally variable composition characteristic of fresh animal tissues. Such uniformity supports more efficient matching of available implantable prosthetic alternatives with the clinical needs of each patient requiring such a prosthesis.

In still another aspect of the present invention, selective and substantially complete removal from animal tissue of one or more of a heterogeneous group of undesired agents also can yield a superior matrix for tissue engineering. Individual cell lines that may be desired in engineered tissue may have differing sensitivities to the various calcific agents commonly found in animal-derived tissues. Thus, methods of the present invention may be used to efficiently tailor tissue engineering matrices comprising tissue of animal origin to encourage growth of particular cell lines and/or to inhibit other cell lines.

Best Mode for Carrying Out the Invention

The supercritical fluid solvent of the present invention is optionally used for treating animal tissues in combination with one or more adjuvants such as cosolvents (e.g., nitrous oxide or ethanol) and/or surfactants (e.g., polysorbate 80 or dipalmitoyl lecithin). Carbon dioxide itself is relatively friendly environmentally, so solvent disposal costs are reduced through its use.

In the present invention, the solvating power of supercritical fluid solvents comprising supercritical or near supercritical carbon dioxide (SCO2) is controlled to cause removal or recovery of selected undesired agents incorporated within or on tissues of animal origin. Note that where more than one undesired agent of the present invention has been dissolved by a supercritical fluid solvent, selective precipitation of each such agent may be obtained through appropriate control of solvent temperature and solvent ambient pressure.

Selective precipitation of undesired agent(s) from a supercritical fluid solvent may be effected, for example, by either heating or cooling such solvents (depending on the solutes incorporated), and/or by decreasing solvent ambient pressure sufficiently to cause reversion of a solvent component to a subcritical state. In preferred embodiments, supercritical fluid solvents comprising SCO2 may further comprise a supercritical cosolvent (such as nitrous oxide). In such embodiments, carbon dioxide and nitrous oxide solvent components may be converted from supercritical to subcritical states simultaneously or sequentially to effect selective recovery of undesired agent(s).

Preheated or precooled portions of recovery apparatus may thus be made to preferentially recover one or more selected undesired agent(s). For example, reduction of solvent temperature

below 36.5 degrees Centigrade will render any nitrous oxide present subcritical, thus generally reducing its solvating power. Similarly, reduction of solvent temperature below 31.0 degrees Centigrade will render any carbon dioxide present subcritical, with an analogous reduction in its solvating power.

It follows that heating or cooling may be selectively used to remove and recover undesired agent(s) from animal tissue. Supercritical fluid solvent introduced by perfusion to preferred locations within and around animal tissue may then selectively remove (that is, transport away, for example, in a dissolved or suspended state) one or more undesired agents. Further, selective recovery (in recovery apparatus) of undesired agents from supercritical fluid solvent previously removed from animal tissue can then be achieved through reduction of solvent ambient pressure which causes precipitation of solute loads substantially in place (that is, without substantial solute redistribution). Establishment of a dynamic solvent ambient pressure gradient within a solvent recovery apparatus, for example, can facilitate selective sequential precipitation of calcific agent(s) at one or more preferred locations within the apparatus.

Although any number of supercritical fluids may be used to achieve a useful biomaterial from which numerous undesired agents have been removed, use of a supercritical fluid solvent comprising carbon dioxide confers several advantages on the methods of the present invention. For example, the viscosity of such a solvent is relatively low, thereby facilitating rapid perfusion of either stabilized or fresh animal tissue. Also, a nonspecific precipitation of undesired agent solutes in a recovery apparatus may be obtained through general reduction of ambient pressure or a sufficiently large solvent temperature reduction. And further, the portion of any supercritical solvent consisting of carbon dioxide is relatively easy to recover and also relatively benign environmentally, thus reducing processing costs.

Disadvantages of using supercritical fluid solvents include the relatively high cost of equipment used to achieve and maintain temperatures and pressures compatible with the corresponding supercritical fluid states of solvent components. Energy costs associated with cycling between subcritical and supercritical states may also be significant. These costs can be reduced, however, if recovery of undesired agents from a supercritical solution is preferably achieved through heating or cooling the solution while it remains in a supercritical state, rather than through converting one or more supercritical fluid solvent components to a subcritical state.

Phrases such as "incorporated in" and "incorporating" as used herein with respect to solvents, animal tissues, or undesired agent recovery apparatus, mean that at least some undesired agent collects in, permeates, adheres to, is transported by, is carried in suspension or solution by, or otherwise becomes associated with the corresponding solvent, tissue, or apparatus. Thus, for

example, an "incorporated" undesired agent may be largely associated with a tissue surface (as, for example, cellular debris), may penetrate within or between the tissue pores, may be covalently or ionically bound to tissue components, may be suspended or dissolved in a supercritical fluid solvent, or may collect in a portion of a recovery apparatus. The nature of the association between undesired agents and the solvent, tissue, or apparatus incorporating them depends on factors such as the particular undesired agent(s) involved, the type(s) of animal tissue, and/or solvent conditions such as temperature, pressure and polarity of cosolvent(s).

An implantable bioprosthetic medical device made and/or used in accordance with the present invention may be selected from any of the numerous device types available to the medical practitioner, including cardiovascular devices, orthopedic implants, and a variety of other prosthetic devices. Examples of such devices may include, but are not limited to, annuloplasty rings, heart valves, catheters, pericardial patches, vascular grafts, wound dressings, sutures, pledgets, and other like devices comprising tissue of animal origin. Additional examples may include femoral prostheses, acetabular prostheses, dental prostheses and the like.

Implantable bioprosthetic medical devices of the present invention may include essentially any implantable medical device comprising tissue of animal origin. These may include medical devices comprised of thermoplastic or polymeric materials such as rubber, plastic, polyethylene, polyurethane, silicone, polytetrafluoroethylene, polyethylene terepthalate, latex, elastomers, and other like materials. These may also include metals (e.g., titanium, cobalt chromium, stainless steel) and ceramics (hydroxyapatite, pyrolytic carbon) in cancellous (i.e., porous) and noncancellous configurations.

The particular embodiments disclosed above are illustrative only, as the invention may be modified and practiced in different but equivalent manners apparent to those skilled in the art having the benefit of the teachings herein. Furthermore, no limitations are intended to the details of construction or design herein shown, other than as described in the claims. It is therefore evident that the particular embodiments disclosed above may be altered or modified and all such variations are considered within the scope and spirit of the invention. Accordingly, protection is sought for the invention disclosed herein as set forth in the claims below.

WHAT IS CLAIMED IS:

1. A treated animal tissue made by a process comprising

providing fresh animal tissue incorporating at least one undesired agent;

providing a supercritical fluid solvent comprising carbon dioxide; and

perfusing said fresh animal tissue with said supercritical fluid solvent to remove at least a portion of said undesired agent from said animal tissue, thereby forming a treated animal tissue.

2. The treated animal tissue of claim 1 made by a process comprising an additional step of stabilizing said fresh animal tissue by crosslinking said animal tissue to provide a stabilized animal tissue prior to said perfusing step.

3. The treated animal tissue of claim 1 or 2 made by a process comprising an additional step just prior to said perfusing step, the additional step comprising

adjusting said supercritical solvent ambient pressure and temperature to achieve a desired solvating power to substantially remove at least one said undesired agent.

4. The treated animal tissue of claim 3 made by a process comprising an additional step just after said perfusing step, the additional step comprising

returning to said adjusting step as necessary for removing substantially all undesired agent from said animal tissue.

5. The treated animal tissue of claim 4 made by a process comprising an additional step just after said returning step, the additional step comprising

separating said supercritical fluid solvent and said retained portion of said undesired agent from said animal tissue.

6. The treated animal tissue of claim 1 or 2 wherein said perfusing step is carried out above a temperature of 45 degrees centigrade.

7. The treated animal tissue of claim 1 or 2 wherein said perfusing step is carried out up to 120 hours.

8. The treated animal tissue of claim 1 or 2 wherein said fresh animal tissue comprises porcine heart valve leaflets.

9. An implantable medical device or tissue engineering matrix comprising the treated animal tissue of claim 1 or 2.

10. The implantable medical device of claim 9, wherein the medical device is a prosthetic heart valve comprising valve leaflets, said valve leaflets comprising said treated animal tissue.

11. A method of estimating calcification tendency of animal tissue incorporating at least one calcific agent, the method comprising

providing a supercritical fluid solvent comprising carbon dioxide;

perfusing said animal tissue with a supercritical fluid solvent;

adjusting the solvating power of the supercritical fluid solvent to selectively incorporate said at least one calcific agent from said animal tissue into said supercritical fluid solvent, thereby forming a calcific solution and a treated animal tissue;

removing said treated animal tissue from said calcific solution;

reducing the solvating power of said calcific solution by lowering the ambient pressure, cooling or heating said calcific solution;

recovering said calcific agent from said calcific solution by filtration or centrifugation; and

estimating calcification tendency of the animal tissue directly proportional to amounts of said at least one calcific agent recoverable.